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To: The Center of Food Safety and Applied Nutrition
5630 Fishers Lane
Room 1061
Rockville, MD 20852

Reference: United States Food and Drug Administration Docket # 91N-0103
"Omega-3 Fatty Acids Lower the Risk of Coronary Heat-t Disease."

Dear Sir or Madam:

As President of The Omega-3 Research Tnstitute, Inc. I respectfully submit the following in strong support of the statement that "Omega-3 (n-3) fatty acids lower the risk of coronary heart disease (CHID)."

My professional background includes 15 years as extramural Director, Metabolic Diseases Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (1978-1992). One area of research covered by the program included fatty acid metabolism in inherited metabolic disorders. As Program Director I was responsible for about 350 grants at academic institutions nationwide. Since I left the NIH in early 1993, I worked as a consultant and focused on the role of omega-3 polyunsaturated fatty acids (PUFA) in health and disease . After a year as Visiting Researcher at the Center of Marine Biotechnology, University of Maryland Biotechnology Tnstitute, Baltimore (1997-1998), I co-founded the Omega-3 Research Institute, Inc. (03RI) where I built a program to provide research grade oils for basic and clinical researchers, and to co-organize workshops and conferences. One such workshop, initiated in collaboration with the Kennedy Krieger Institute, Baltimore, Maryland, is an International Workshop on Brain Uptake and Utilization of Fatty Acids: Application to Peroxisomal Biogenesis Disorders, March 2-4, 2000, Bethesda, Maryland.

Submission of the attached material is intended to exemplify the breadth and variety of approaches used in basic and clinical studies/trials that resulted in similar outcomes, i.e., "Omega-3 fatty acids

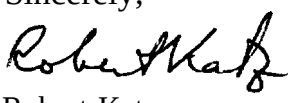
lower the risk of coronary heart disease." This submission does not contain a rigorous scientific evaluation of both sides; rigorous scientific evaluation by itself is only one component of the equation used in reaching public health decisions. Health benefits to the public and potential risks and safety considerations are also important factors in reaching such decisions.

For example, according to the American Heart Association, about 500,000 Americans will suffer some kind of heart disease each year. Even a 10% reduction in that number would amount to 50,000 healthier and functional individuals whose deaths would be avoided and morbidity expenses saved.

Each one of the three clinical studies/trials discussed below reported at least a 10% reduction in the relative risks of recurring fatal myocardial infarcts (Ml) non-fatal (N-F) myocardial infarcts and occurrence of other death and N-F strokes.

In addition we discuss the issues of the Recommendation for Adequate Intake (AI) of omega-6 and omega-3 fatty acids as supported by prior clinical trials and conclude with a summary description of the inverse relationship of omega-3 consumption and levels of triglycerides in plasma. These studies suggest an inverse association between omega-3 fatty acids and lowering the risk of coronary heart disease.

Sincerely,



Robert Katz
President

Omega-3 PUFA in Clinical Trials/Studies with Coronary Heart Disease (CHD) Risk Factor-Related Endpoints.

The majority of basic and clinical studies as well as several clinical trials using various types of omega-3-rich foods or supplements[1-10] strongly suggest that there is a negative correlation between omega-3 (n-3) consumption and risk of coronary heart disease.

Research showed that n-3 polyunsaturated fatty acids (PUFA) displayed potential beneficial effects on cardiovascular risk factors such as antiatherogenicity, antithrombotic and antiarrhythmic [11-13] and hypotriglyceridemic' effects. The Chicago Western Electric Study[5] (Chicago), entitled "Fish consumption and the 30-year risk of fatal myocardial infarction"; the GISSI-Prevention Trial" (GISSI); entitled "Dietary supplementation with n-3 PUFA and vitamin E after myocardial infarction: results of the GTSSI-Prevenzione trial" and the Lyon Diet Heart Trial[8] (Lyon), entitled "Mediterranean diet , traditional risk factors and the rate of cardiovascular complications after myocardial infarct" are three representative studies discussed below. Table 1 summarizes the structure, organization and outcomes of the Chicago, GISSI and Lyon studies.

Comparison of the Chicago Western Electric Study, the GISSI-Prevention Trial and the Lyon Diet Heart Trial

TABLE 1

Chicago	GISSI	Lyon
	Design and Nature	
Single center	Multicenter (172)	Single center
Stratified	Randomized	Randomized
Observational	Interventional (secondary prevention)	Interventional (secondary prevention)
Open	Open (oversight committee blinded)	Single blind
Retrospective	Prospective	Prospective

Table 1, contd.

Patients		
1,822 Free of cardio-vascular disease (CVD)	11,324 Myocardial infarct (MI) <3 mo	605 MI (recovered) , 6 mo.
Have all required data on diet, confounding variables, & CHD risk factors	No unfavorable short-term outlook	No heart failure. To be clinically stable and ambulatory. Ability to perform a specific exercise test.
40-45 years of age	No defined age	<70 years old
Under regular medical supervision	Remained on prescribed medication	Remained on prescribed medication
Length of Follow-up Period		
30 years	3 to 5 years	-3.8 years
Study and Trial Endpoints		
Primary Death from CHD Death from CVD Death from MI	First primary endpoint analysis group: Death (total for all reasons) Non-fatal MI (recurring) N-F stroke Second primary endpoint anal. group: CV death, N-F MI and N-F stroke	Primary Cardiac death & Non-fatal (N-F) Ml (CO 1)

Table 1 contd.

Treatment Groups and Therapy Design

Stratified cohorts on diet containing	Equal cohorts of randomized patients:	Equal cohort of randomized patients:
1. >35.0 g fish/day	1. Fish oil supplemen. group (1 .O g EPA + DHA/day)	1. Experimental group [with diet modeled after the Cretan diet (Mediterranean) high in oleic acid and alpha-linolenic acid (LNA,18:3n-3)]
2. 18 to 34 g. fish/day	2. Fish oil & vitamin E supplemented group (1 .O g EPA & DHA/day and 300 mg vitamin E/day)	2. Control group (with regular physician-recommended diet)
3. 1 to 17 g. fish/day	3. Vitamin E supplementation group (300 mg/day)	
4. 0 g. fish/day (controls)	4. No supplementation group (controls) and regular diet	

Statistically Significant Outcomes (Primary Endpoints)

Relative Risks:	Relative Risk:	Risk Ratio
1. 0.56 (>35 g fish consumed) (Fatal CHD & CVD)	1. 0.90 (Fish oil only) (Death,N-F MI & N-F stroke)	1. 0.28 (CO 1) (Cardiac death & N-F MI)
2. 0.76 (18-34 g fish) (Fatal CHD & CVD)	2. 0.86 (Fish oil & vitamin E) (Death, N-F MI & N-F stroke)	
3. 0.88 (l-17 g fish) (Fatal CHD & CVD)		

Adjustment of Outcomes

Outcomes have been adjusted for CHD risk factors and confounding variables such as age, religion, systolic blood pressure, serum cholesterol, smoking, body mass index, diabetes, daily intake of energy, ethanol, some macro- and micronutrients, etc.

Major differences and similarities among the three studies in Table 1 are noted below:

Major differences:

1. Nature of the study or clinical trial, e.g., observational (Chicago) *vs*, interventional (GISSI and Lyon); stratified (Chicago) *vs*. Randomized (GSSI and Lyon); single center (Chicago and Lyon) vs. Multicenter (GSSI); retrospective (Chicago) vs. prospective (GSSI and Lyon).

2. The observational study (Chicago) had a 30-year long follow-up period vs. the interventional trials where therapy and follow-up lasted between 3 and 5 years.

3. Omega-3 PUFA was administered to the appropriate treatment groups in three different forms:

   a. As amount of fish consumed per day (Chicago)
   b. As supplemented fish oil in 1 .O g per day capsules containing eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in a 2: 1 ratio (GISSI)
   c. As a special Mediterranean diet (MedDiet), (Lyon) that was enriched in alpha-linolenic acid (ALN, 18: 1 n-9) and oleic acid (18: 1 n-9) the major component of olive oil.

In the Chicago study two nutritionists collected the information at the first and second examinations, 1 year apart with standard questionnaires on typical workday and weekend day eating patterns. Fish consumption was assessed as part of the overall eating pattern, and a profile was established that contained some 26 foods over a 28 day period. The food profile included soft drinks; coffee; milk (whole and skim); cream, cheese; eggs; ice cream, puddings or custards; soups; fish, beef; veal or lamb; pork; ham or bacon; liver; poultry; vegetables; breads and cereals; potatoes; fruits; pastries; sugar; butter; margarine and fried foods. Items were coded on a four point scale as 0 for none, 1 for low, 2 for moderate and 3 for high consumption. Fish consumption in 120 g units per 28 days was coded as 0 for none, 1 for less than 4 units, 2 for 4 to 8 units and 3 for more than 8 units. The profile obtained was a representative cross section of a Mid-Western American diet. The average omega-6 and omega-3 profiles of an American diet as assessed by an ongoing food survey of the U.S. Department of Agriculture for the 1994- 1996 period, presented in Table 2, indicates that the ratio of omega-6 to omega-3 PUFA in a daily food intake is about 10: 1. It also shows that the major polyunsaturated fat consumed by the American public is linoleic acid (LA, 18:3n-6) and that the daily intake of long-chain n-3 PUFA (EPA and DHA) is negligible. Given these intake levels, the study outcome of the cohort that consumed >35 g fish/day becomes even more significant. It should be noted that the 10:1 ratio for omega-6:omega-3 intake should be considered a low value. Larger intake of LA and lower intake of LNA, would raise this ratio even higher. A discussion of the association between high values for the omega-6:omega-3 ratio and increased risk of CHD, later in the Lyon study, will further clearify this issue.

Intake of Individual Fatty Acids
Food Surveys Research Group
Beltsville Human Nutrition Center
United States Department of Agriculture*

TABLE 2

Mean percentages of food energy, 1 Day

Sex and Age	Food Energy Intake	18:2	18:3	18:4	20:4	20:5	22.5	22.6	Total PUFA
All Indiv.	2,002 kcal	5.7	0.6	*	0.1	*	*	*	6.4

Omega 6: Omega-3 ratio 9.6: 1
*Values less than 0.05 but more than zero.

Mean percentages of PUFA intakes (in grams)

All Indiv.		12.9	1.3	*	0.1	*	*	*	14.6

Omega 6: Omega-3 ratio 10: 1

Mean percentages of total contributed by individual fatty acids

All Indiv.		19.0	1.9		0.2	0.1		0.1	

Omega 6: Omega-3 ratio 10: 1

* Results from the 1994- 1996 Continuing Survey of Food Intakes by Individuals. The data provides national probability estimates for the U.S. population (over 16,000 individuals participated in a survey known as What We Eat in America). Recall data represent weighted intakes based on respondents first of 2 days reported.

The investigators of the GISSI trial assessed the patient's dietary habits with questionnaires. Fish (>1 serving/week), fruit (<1 serving/day), fresh vegetables (>1 serving/day) and olive oil consumption were followed every 6 months during the trial. The regimen of one capsule/day of fish oil containing about 850 mg of n-3 PUFA (EPA and DHA as ethyl esters in a 1:2 ratio) used in the trial was considered similar to a diet that contained about 100 g of fatty fish consumed every day, This amount is significantly lower than the pharmacological doses of fish oil used in mechanistic and animal studies. The moderate fish oils provided daily were considered in line with a need for long term supplementation. Although the value of the omega-6: omega-3 intake is

not provided for this trial, it should approach the value of the typical Japanese diet (between 4: 1 and 2: 1).

The MedDiet in the Lyon trial was modeled after the Cretan Diet discovered in the Seven Countries Study[14]. In this study, a 5-l 5 year follow-up concluded that, mortality rates from CHD in Southern Europe was two-to-threefold lower than in Northern Europe or the United States [15, 16] (see Table 3). It is interesting to note that in the Seven Countries Study the cohort from Crete presented a CHD mortality that was 95% less than in Northern European Countries and 98% less than the U.S. cohort. It is also of interest to note that the differences in cholesterol levels do not appear to account for these large differences in mortality by themselves.

Thus the diet for the experimental group of the Lyon Diet Heart Trial was constructed to contain a) more bread, b) more vegetables and legumes, c) more fish, d) less meat (beef, lamb, pork) and replaced with poultry, e) no day without fruit and f) no butter and cream. Butter and cream were replaced with a special, erucic acid-free canola oil-based margarine comparable with olive oil (high in oleic acid, 18: ln-9) enriched mostly with alpha-linolenic acid (LNA, 18:3n-3) and slightly with linoleic acid (LA, 18:2n-6). Its composition is 8.4% 16:0 (saturated fat), 6% 18:0 (saturated fat), 48% *cis* 18:1n-9, 5.4% *trans* 18:1n-9, 16.4% *cis* 18:2n-6 and 4.8% *cis* 18:3n-3. This margarine, which was used for food preparations and on bread contains an omega-6:omega-3 ratio of 3: 1. Oils recommended for salads and food preparations were erucic acid-free rapeseed oil (relatively high in 18:3n-3) and olive oil (high in 18:1 n-9). Moderate amounts of alcohol in form of red wine were allowed and recommended at meals. Counseling by dietitians was provided, and compliance with the diet was monitored by providing measured amounts of margarine at outpatient clinic bimonthly visits and by checking consumption at each visit to the clinic. Dietary surveys were conducted regularly through 24 hour recall and a food frequency questionnaire. The study showed that the approximately 300 patients randomized to the experimental group on the MedDiet maintained their diets during the average of 46 months follow-up period.

Fatty acid analysis of plasma lipids was performed at each visit. After only 2 months of trial (at the first follow-up) plasma fatty acid levels of the experimental group showed statistically significant increases in 18: ln-9, 18:3n-3, 20:5n-3 and 22:6n-3 and decreases in 18:0, 18:2n-6 and arachidonic acid (20:4n-6, another prevalent and important omega-6 PUFA) levels vs. the control group. These changes brought about a lowering of the n-6:n-3 ratio in plasma of the experimental group vs. the control group from 11.6 to 9.95 during the same period.

Seven Countries Study: Comparison of Selected Cohorts

Table 3.

	Crete	Mediterranean	Zutphen [4], Netherlands	u s Railroads
Mortality				
Total	514	1090	1091	1153
CHD	9	184	420	574
Cholesterol (mmol/L)	5.3	5.0	6.0	6.1
Foodstuff (g/d)				
Bread	380	416	252	97
Legume	30	18	2	1
Fruit	464	130	82	233
Meat	35	140	138	273
Fish	18	34	12	3
Edible fat	95	GO	79	33
Alcohol	15	43	3	6

From Renaud S. et al.,'"

Major similarities

1. Patient selection criteria were comparable in all three studies.

2. Primary endpoints are comparable. These included death from all causes, death due to MI, to CVD, to CHD, N-F, MI and N-F stroke.

3. Relative risks and risk ratio outcomes show reduced recurrence or occurrence of primary endpoints in all studies.

In the Chicago study all-cause and cardiovascular mortality and the combination of recurrent MI and cardiac death (CO1) were reduced (risk ratio for CO1 was 0.28).

In the GISSI trial it was shown that the magnitude of effectiveness of the n-3 PUFA treatment on occurrence of total death, N-F MI and N-F Stroke (combined primary endpoints) corresponds to a 10% relative decrease in **risk** in the 2-way analysis (considering only the cohorts supplemented with fish oil vs. the controls).and 15% relative decrease in the 4-way analysis (considering all supplemented cohorts and controls).

The magnitude of effectiveness of the n-3 PUFA treatment on occurrence of cardiovascular death, N-F MI and N-F Stroke (combined primary endpoints) reaches a 20% relative decrease in the 4-way analysis [showing the more realistic ("true") results].

It should be noted that Vitamin E supplementation had no added advantages for the primary endpoint groups analyzed. The Italian subjects included in the GISSI trial could have already been on a Mediterranean-type diet. Thus, the 300 mg vitamin E would not have had the same beneficial effect on CHD risk factors such as inhibition of LDL-cholesterol oxidation, in this population as was found in other studies.'"

Since the subjects in all groups were treated with various drugs, including aspirin, the observed preventive outcomes for n-3 PUFA supplementation are largely independent and above those effected by the drug therapies.

The beneficiary effect of EPA and DHA in the cardiac signal and transduction pathway and its potential of reducing the incidence of instant cardiac death from ventricular fibrillation has been extensively documented in recent studies by billman et al.,".

The Lyon Heart Diet Study [8,16], with its diet based on increased consumption of bread and cereals, vegetables and legumes, fruit, fish and edible vegetable fat, resulted in an adjusted, decreased risk of recurrence ratio of 0.28 (95% CI, p=0.0001), a highly significant reduction in combined cardiac death, and N-F MI in favor of the experimental group on the MedDiet in the CO 1 endpoints group.

Although the Lyon study did not find any correlation involving long-chain omega-3 PUFA (EPA or DHA) the study design did not emphasize their role in the Mediterranean diet. It should be noted, however, that the levels of alpha-linolenic acid EPA and DI-IA in plasma lipids increase in the experimental group, while the levels of n-6 acids (linoleic and arachidonic 20:4n-6) decrease. Dietary linolenic acid is the precursor of EPA and DHA *in vivo*. Nevertheless, given the slow rate and low yield of the 18:3n-3 to 20:5n-3 *in vivo* transformation steps, both EPA and DHA can be supplemented by inclusion of fatty fish and some seafood varieties in diet by preparing food products with supplemented EPA and DHA or by taking moderate amounts of concentrated omega-3 oil supplements.. Alpha-linolenic acid (18:3n-3) was associated with improved prognosis in agreement with another recent study outcome [17].

4. Adjustment of outcomes for CHD risk factors or confounding variables is comparable among the studies. These include plasma cholesterol levels (LDL) and total cholesterol, systolic blood pressure, blood leukocyte counts, smoking and cardiovascular medication.

Conclusions

1. Varied approaches utilized by the Chicago study, and by the GISSI and Lyon trials resulted in comparable endpoint outcomes, all pointing strongly toward an inverse relationship between omega-3 in the diet and reduced risk of CDH,

2. It appears that a modified Mediterranean diet (MedDiet), modeled after a diet from the island of Crete and based on high oleic and alpha linolenic acid content, provides increased protection from CHD,

3. In line with the original study of Bang, Dyerberg, and Hjorne [20], who reported for the first time a possible connection between the almost complete lack of CHD among the Greenland Eskimos and the high concentration of long-chain n-3 PUFA (EPA and DHA) that their diet contained, the Chicago study and the GISSI trial showed that high level fish consumption (>35 g/day) and supplementation of EPA and DHA (about 850 mg/day as EPA and DHA in a 1:2 ratio) are both beneficial in reducing the occurrence or recurrence of fatal and non-fatal coronary events.

4. The above clinical studies and trials also pinpoint the importance of a low omega-6 to omega-3 ratio in the maintenance of a healthy cardiovascular system.

5. Although we are still far from understanding the mechanisms through which omega-3 fatty acids exert their positive effects on lowering the risk of CHD, the amassed clinical information justify the acknowledgement of their positive effects in protecting the cardiovascular health of American public at large.

6. Although there were no significant deleterious or toxic effects reported in these trials, long-term administration of pharmacological amounts of fish oils should be considered only under supervision of a physician.

Recommendations for Adequate Intakes (AI) of PUFA for Adults

Participants of the NIH Tntemational Workshop on the Essentiality of, and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids presented and discussed clinical nutritional study outcomes in the established fields of infant nutrition and cardiovascular disease and the new and promising area of mental health. The international nature and outstanding professional level of the participants rendered the group and its conclusions highly competent.[21,22] A summary of the recommendations for adults is presented in Table 4..

The group agreed that sufficient scientific evidence has been amassed in the infant nutrition and cardiovascular areas to recommend daily adequate intakes (AIs) of omega-6 and omega-3 essential fatty acids for adult diets and for infant formulas/diets. The group also discussed the

health effects of trans-fatty acids and recommended an upper limit for daily AI of trans-fatty acids in adults, In a similar fashion, an upper limit for saturated fatty acids was recommended for adults.

Table 4, AIs for Adults, details the recommendation for linoleic acid (LA) (or 18:2n-6, the essential polyunsaturated omega-6 fatty acid) for alpha-linolenic acid (LNA) (or 18:3n-3, the essential polyunsaturated omega-3 fatty acid), and for eicosapentaenoic acid (EPA 20:5-n-3) and docosahexaenoic acid (DHA 22:6n-3), which are the penta- and hexapolyunsaturated long chain omega-3 fatty acids derived through metabolic processes from LA.

The group consensus was that due to the low yield of the LNA to EPA to DHA pathway, it is advisable to supplement EPA and DHA resulting from the above pathway with naturally occurring EPA and DHA (from fish, other foods, or nutritional supplements). Tt should be noted that beside LA, the group did not recommend AIs for arachidonic acid (AA, 20:4n-6), a metabolic transformation product of LA. The reason for this is twofold: 1) The LA to AA pathway provides higher yields of elongated and further desaturated products, and 2) In the general population the direct consumption of AA is much higher than EPA or DHA since AA is one of the major polyunsaturated fatty acids in red meat (beef, lamb, or pork).

In terms of daily AIs recommended for LA (upper limit) vs. LNA+DHA and EPA, in other words the omega-6:omega-3 ratio, is 2.3: I. Even if AA consumption is high (2.0 to 3.0 g/ day),(limited by the recommended 8.0 g upper limit for saturated fats with which AA is associated in red meat), this ratio will not climb above 3.36: 1 The omega-6:omega-3 ratio in adult plasma varies between 10.0 and 27.0, indicating that the major source of fat consumed in today's Western diet is LA. Hence, the recommendation that monosaturated fatty acids constitute the majority of fatty acids consumed each day.

Adequate Intakes (AI) of PUFA for Adults*

Recommendations of an Expert Working Group (WG) on Omega-6 and Omega-3 PUFA Intakes.

TABLE

Fatty Acid	Grams/day	% Energy
LA (linoleic acid) Upper-limit	4.44 6.67	2.0 3.0
LNA (alpha-linolenic acid)	2.22	1.0
DHA & EPA DHA to be at least* EPA to be at least*	0.65 0.22 0.22	0.3 0.1 0.1
Trans-FA** (upper-limit)	2.00	1.0
Saturated (upper-limit)	———	<8.0
MONOS***	———	———

*For pregnant and lactating women ensure 300 mg/d of DHA

**Except for dairy products, other foods under natural conditions do not contain trans-FA.
Therefore the WG does not recommend trans-FA to be in the food supply.

***The WG recommends that the majority of FA ingested be from monounsaturates.

The recommendations of Table 4 converge on the elements of a modified Mediterranean diet. Such a diet was also described in a recently published book entitled *The Omega Plan* [23].

The Hypotriglyceridemic Effect of Long-Chain Omega-3 Fatty Acids

The GISSI Prevention trial [10] reported a statistically significant lowering of triacylglyceride (TAG) levels in the plasma of the subjects supplemented with fish oils. Several other studies indicate an inverse relationship between plasma TAG concentrations and omega-3 consumption.

A few examples of n-3 PUFA clinical supplementation studies in triglyceridemic subjects are mentioned below:

1. A review [24] of 72 placebo-controlled human studies which supplemented a range of 1 .O to 7.0g. EPA and DHA daily for at least 2 weeks reduce plasma TAG concentrations by 25-30%.

2. A background diet rich in n-3 PUFA reduced postprandial response to a saturated fat test meal in comparison with response to a saturated fat meal lacking the n-3 PUFA-rich background diet[25,26].

3. Using lower doses of n-3 PUFA for longer times resulted in an equivalent hypotriacyglycerolemic effect [27,28]

4. 7.0g/d n-3 PUFA significantly reduced fasting plasma TAG concentration by 21.2% and the postprandial TAG response by 32% [29]. (This shows that dietary intake of *n-3* PUFA as a supplement has a positive effect on TAG metabolism). [29]

The above data strongly suggests the possibility of an inverse relationship between n-3 PUFA and risk of CHD.

List of Refcrcnces

References on n-3 PUFA basic and clinical studies/trials

1. Burr, M.L., Fehily, A.M., Gilbert, J.F., et al.; Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART), *Lancet* 1989,ii: 757-61.
2. Ascherio, A., Rimm, E.B., Stampfer, M.J., et al.; Dietary intake of marine n-3 fatty acids, fish intake and the risk of coronary disease among men (The Health Professionals Study),*N Engl J Med* 1995; *332: 977-982.*
3. Albert, C.M., Hennekens, C.H., O'Donnell, C.J, et al.; Fish consumption and risk of sudden cardiac death (U.S. Physicians' Health Study), *JAMA* 1998; 279: 23-28.
4. Kromhout, D., Bosschieter,E.B., de Lezenne;. The inverse relationship between fish consumption and 20-year mortality from coronary heart disease (Zutphen Study), *N Engl J Med* 1985; 3 12: 1205-1209.

5. Daviglus, M.L., Stamler, J., Orencia, A.J., et al.; Fish consumption and the 30-year risk of fatal myocardial infarction (Western Electric Study), *N Engl J Med* 1997; 336: 1046-53.
6. Dolecek, T.A.; Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention Trial *Proc, Soc Exp Biol Med* 1992; 200: 177-182.
7. Rodriguez, B.L., Sharp, D.S., Abbott, R.D, et al.; Fish intake may limit the increase in risk of coronary heart disease morbidity and mortality among heavy smokers: The Honolulu Heart Program *Circulation 1996; 94: 952-956.*
8. DeLorgeril, M., Salen, P., Martin, J-L, et al.; Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study *Circulation 1999; 99: 779-785.*
9. Singh, R.B., Rastogi, S.S., Verma, R., et al.; Randomised controlled trial of cardioprotective diet in patients with recent acute myocardial infarction: results of one year follow **up** (The Indian Trial), *BMJ* 1992; 304: 1015-19.
10. Marchioli R., et al., Dietary Supplementation with n-3 polyunsaturated Fatty Acids and Vitamin E After Myocardial Infarction: Results of the GISSI-Prevenzione Trail. *Lancet*, 1999; 354: 447-455.
1 1. Simopoulos, AP., Omega-3 fatty acids in health and disease and growth and development: *Am J Clin Nutr* 1991;54: 438-53
12. Simopoulos, AP.; N-3 fatty acids in the management of cardiovasucular disease. *Can J Physiol Pharmacology* 1997;75: 234-39
13. Marchioli; R., DiPasquale, A., per: Ricercatori GISSI-Prevenzione G *Ital Cardiol* 1993;23: 933-69.
14. Keys, A., Coronary Heart disease in seven countries. *Circulation* 1970; 4 1 (suppll): 1-2 11.
15. Keys ,A., Menotti A, Aravanis C, et al.; The Seven Countries Study: 2289 death in 15 years. *J. Prev Med* 1984; 13:141-54.
16. Renaud S., DeLorgeril et al., Mediterranean alpha linolenic acid-rich diet in the secondary prevention of coronary heart disease. *Lnncet.* 1994; 343: 1454-9.
17. Ascherio A., et al.; Dietary fat and risk of coronary heart disease in man: cohort follow **up** study in the United States. *BMJ* 1996; 3 13: 84-90.
18. Billman G.E., Kang J.X., Leaf A., Prevention of Ischemia-induced cardiac sudden death by n-3 polyunsaturated fatty acids. *Lipids* 1997; 116 1 - 1 168 and references therein.
19. Princen, H.M.G., van Duyvenvoorde, W., Brytenhek, R., et al.; Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women, *Artherioscl. Throm. Biol*, 1994, 15(3): 325-32.
20. Bang, H.O., Dyerberg, J., and Hjorne, N.; The composition of foods consumed by Greenland Eskimos, *Acta Med.* Scand. 1976; 200: 67-73.
21. Simopoulos, A.P., Leaf, A., Salem, N. Jr.; Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann Nutr Metab* 1999; 43: 127-l 30.
22. Simopoulos, A.P., Leaf, A., Salem, N. Jr.; Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Food Australia* 1999; 5: 332-333.
23 The Omega.Plan. A.P.Simopoulos and Jay Robinson. HarperCollins, N.Y., 1889.
24 Harris, W.S., n-3 Fatty acids and lipoproteins: Comparison of results from human and animal studies. *Lipids,* 1996; 3 1: 243-252.

25. Harris, W.S., Connor, W.E., Alam, N., and Tllingworth, R.D., Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. *J. Lipid* Res 1998; 29 1: 145 l-60
26. Weintraub, M.S., Zechner, R., Brown, A., Dietary polyunsaturated fats of the W-6 and W-3 series reduce postprandial lipoprotein levels. et al., *J Clin Invest* 1988; 82: 1884-93.
27. Williams, C.M., Moore, F., Morgan, L., and Wright J., Effects on n-3 fatty acids on postprandial triacylglycerol and hormone concentrations in normal subjects. *Br J Nutr 1992; 68: 655-66.*
28. Agren JJ, Hanninen 0, Julkunen A, Fogelholm L, Vidgren H, Schwab U, Pynnonen 0 & Unsitupa M. Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels.
29. Roche, H.M., and Gibney, M.J., Eur J Clin Nutr 1996; 50: 617-24.